Body-size evolution: **How to evolve a mammoth moth**

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Separate recent studies have revealed the physiological changes underlying the evolution of body size in an insect and advanced our understanding of the genetics of insect growth. These studies highlight the gulf between physiological and genetic studies of growth control and the exciting opportunities for unification of these fields.

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What makes rats larger than mice and butterflies larger than flies? Body size is a central parameter in evolution and ecology. For example, body size is a crucial factor in predator–prey interactions and mate choice. Although change in body size is the most pervasive pattern in evolution, the evolution of the mechanisms controlling body size remains largely unexplored. The final size of an adult results from an interaction of genetic and environmental factors, for example intrinsic growth rates and food availability. Whilst the quantity of food available to a growing organism is an obvious determinant of final body size, it is clearly not the only determinant. Body size is also controlled by genetic factors, as illustrated by the fact that individuals of a single species tend to be about the same size: mice are small, rats are larger and elephants are huge.

To date, physiologists and geneticists have taken distinct approaches to this problem, leading to surprisingly different kinds of understanding of the control of body size. Physiological studies of growth control have focused on determining the role of hormones in controlling the timing of critical events in the growth of an organism, and we have the most detailed understanding of growth control in insects. To a first approximation, the final size of an insect is simply the product of its growth rate and the duration of growth. As insects tend to grow exponentially, the duration of growth is a crucial parameter, which is typically controlled by the secretion of specific hormones in response to the organism reaching particular milestones.

In contrast, geneticists have ignored the details of growth rate and the duration of growth and simply searched for genes that, when affected by mutation, result in larger or smaller organisms. This search has led to the discovery that the insulin receptor pathway plays a crucial role in growth control in *Drosophila*. In addition, detailed developmental genetic studies have revealed that the final size of a fly arises largely independently of the specific parameters of cell growth and proliferation. Body size is controlled above the level of individual cells by a mechanism that integrates genetics and physiology. Our studies must do the same.

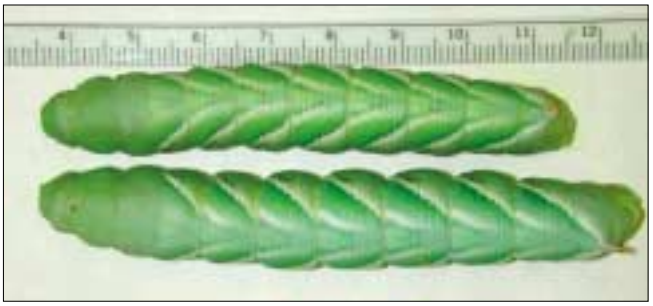
The evolution of body size by physiological evolution

Holometabolous insects, which undergo complete metamorphosis during a pupal stage, provide powerful systems for dissecting the control of growth because adults do not grow and body size is determined by the size of the larva when it pupates. The tobacco hornworm *Manduca sexta* — actually a moth — is one of the few organisms for which the physiological mechanisms that regulate final body size are well understood [1]. Larvae increase in size at an approximately exponential rate, so that 88% of larval growth occurs in the last instar. This has important consequences. The final size of a larva is obviously dependent on its growth rate, but less obviously determined by a series of physiological events related to the attainment of a 'critical weight' — the size at which a larva becomes irreversibly committed to pupating.

Approximately midway during the last larval instar, the attainment of this critical weight causes, by unknown mechanisms, a drop in the circulating levels of juvenile hormone. When juvenile hormone is removed from the haemolymph, the larva becomes competent to secrete prothoracicotropic hormone (PTTH). Although larvae become competent to secrete PTTH after juvenile hormone levels fall, they only secrete PTTH during a particular time window of each day, the 'photoperiodic gate'. Secretion of PTTH stimulates the production of ecdysteroids, which cause the larva to stop feeding and trigger the final commitment to pupation. The critical weight is not simply a genetically determined size, but instead is a genetically influenced function of the size of the larva at the beginning of the final instar: larvae that are larger at the beginning of the final instar have a larger critical weight [2].

There are therefore five processes that determine adult size. The first mechanism is the size of the larva at the beginning of the final instar, as this determines the magnitude of the critical weight. The second is the growth rate during the final instar, as this not only allows attainment of the critical weight faster (which alone would not alter body size), but more importantly allows faster growth after the attainment of the critical weight but before the secretion of ecdysteroids. Third is the genetic component of the critical weight. Fourth is the time from the attainment of the critical weight until all of the juvenile hormone is

Figure 1



Manduca sexta fifth-instar larvae representative of the 1970 (top) and 1999 population (bottom). Larvae from the evolved population are almost 50% heavier than those from the original population. (Photograph courtesy Goggy Davidowitz.)

cleared from the haemolymph, which allows the secretion of PTTH. And fifth is the duration of the photoperiodic gate allowing PTTH secretion. (If the duration of the photoperiodic gate were to decrease, then larvae that became competent to secrete PTTH at random times during the day would have to wait longer, on average, before they could secrete PTTH, thereby increasing average body size.) These mechanisms have been well understood for several decades.

In their recent study, D'Amico *et al.* [1] have provided our first glimpse of how these mechanisms can evolve to generate a large change in the body size of an insect, and the results have provided several surprises. They took advantage of the fact that a laboratory population of *Manduca sexta* has evolved to be 50% larger than the founder population which was established in the late 1960s (Figure 1). Fortuitously, this population served as the subject of detailed studies on the developmental control of body size in the early 1970s. D'Amico *et al.* [1] simply repeated these studies on the contemporary population. Perhaps the biggest surprise was that they found no change in the size of the larvae at the beginning of the final instar. This was a surprise because a small change in size at the beginning of the instar would result in a large increase in final body size without requiring any other changes in developmental mechanisms. Evolution seems not to have taken what we might intuitively think of as the easiest path, and it will be interesting to determine why this might be.

Instead, D'Amico *et al.* [1] found that the 1999 population of *Manduca sexta* had a greatly elevated growth rate in the final instar. This suggests, surprisingly, that the growth rate in the early instars may be under different genetic control than growth in the final instar. In addition, they found that the value of the critical weight had increased in the 1999 population and that the delay between the attainment of critical weight and the secretion of PTTH had increased. They found that the duration of the photoperiodic gate had not evolved. Therefore, three of the five mechanisms controlling final body size had evolved. Even more impressive, the observed changes in the final-instar growth rate, the critical weight and the delay to PTTH secretion explain more than 95% of the change in final body size.

These results illustrate that developmental mechanisms that are often considered species-specific traits actually contain genetic variability within natural populations (a prerequisite for their evolution, of course). Different species may therefore evolve convergent body sizes through different mechanisms, and comparative studies are required to unravel the universe of potential solutions to the problem of growth control. At the moment, however, an even larger problem looms: genetic and physiological studies of growth control appear to have no regions of intellectual overlap.

Genetic studies of growth control

Studies of the genetic control of growth have been the subject of several recent reviews [3–5] and here I shall only briefly summarise the major findings. Although a variety of mutations in *Drosophila* cause changes in body size, one pathway in particular, the insulin receptor pathway, has attracted considerable attention because defects in this pathway cause clear effects on the final size of the fly. Under-activation of the pathway generates small flies, whereas over-activation generates large flies [6]. *Drosophila* contains seven insulin-like genes and Brogiolo *et al.* [6] recently demonstrated that over-expression of one of these genes causes overgrowth in an insulin-receptor-dependent fashion.

The major gap in our understanding is what role the insulin receptor pathway plays during natural growth of the fly. Is the insulin receptor pathway simply required for normal growth, or does it play the more interesting role of responding to environmental variation, particularly variation in nutrition, to modulate final body size? It is this type of information that might allow us to begin connecting the genetic and physiological studies. For example, does the insulin receptor pathway regulate growth rate or critical size, both or neither? Current evidence suggests that the insulin receptor pathway acts autonomously on cells within growing organs. It is not yet clear how this type of cell-autonomous regulation relates to the more systemic regulation revealed by physiological studies, unless growing organs are somehow signalling to the endocrine organs once they attain a particular size. This mechanism would require first that the organs themselves 'know' their target size, and second that they could signal to the endocrine organs once they had attained this size.

There is evidence that such a mechanism operates in insects [3], and this may help connect the genetic and physiological sides of growth. First, final organ size in *Drosophila* is not a simple product of cell growth and proliferation, but instead is determined by interactions across a tissue. Many experiments illustrate this point, but the earliest evidence came from studies of somatic clones that were caused to overproliferate [7,8]. Growth of these cells might be expected to cause cancerous overgrowths. But instead the resulting tissues are perfectly normal in pattern, shape and size, showing that the specific patterns of cellular proliferation within tissues are irrelevant to final size.

Similarly, several studies point to a role for growing tissues in regulating the physiology of insect growth. For example, when the growing tissues that will generate the adult structures — the imaginal discs — are damaged, their regrowth causes a delay of the final moult until the tissues grow back to their normal size [9]. In addition, in flies carrying mutations that cause overproliferation of imaginal discs, larvae continue to feed and grow larger than their normal size [10]. The overproliferation itself causes the delay in pupation (rather than the other way around), suggesting that the critical size in *Drosophila* is actually dependent on the cessation of proliferation within imaginal discs, and perhaps that the critical size is actually regulated by imaginal discs (see Stern and Emlen [3] for a review of several other relevant experiments). This might explain why it has proven so difficult to isolate 'the' mechanism of critical-size determination in most insects [11]; critical size may be the product of a community of growing tissues signalling to the endocrine system that they have reached a suitable size. If so, then the insulin receptor pathway may regulate final size of the whole organism simply by autonomously regulating organ size.

One final example may provide further clues to the connection between physiological and genetic mechanisms of growth control. In the silkmoth *Bombyx mori* a large family of insulin-like proteins, the bombyxins, has been discovered [12]. These proteins are expressed in the brain as well as several other tissues, like the insulin genes of *Drosophila* [6], and in the saturniid moth *Samia cynthia* they are capable of inducing the prothoracic gland to secrete ecdysteroids [13]. (Strangely, the bombyxins do not possess prothoracicotropic activity in *Bombyx*, although there is evidence that the two species possess different prothoracicotropic hormones.) The physiological role of bombyxins is not yet clear, nor is their evidence that these insulin-like proteins can also control growth, but the prothoracicotropic activity of these peptides provides a potential linkage between insulin signalling and the physiological control of growth. Thus, insulin-like molecules contribute directly to control of growth, as demonstrated in *Drosophila*, and perhaps to control of the timing of developmental events. A large challenge for the future is to marry genetic and physiological studies of growth to unravel the apparently complex role of genes, such as those involved in insulin signalling, in the control of growth. Only then will a truly integrative understanding of body size evolution be possible.

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